REPLACEMENT OF FISHMEAL WITH PLANT FEEDSTUFFS IN THE DIET OF RED DRUM *SCIAENOPS OCELLATUS*: AN ASSESSMENT OF NUTRITIONAL VALUE

A Thesis

by

JOSEPH DALE MOXLEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2012

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Chair of Committee,	Delbert M. Gatlin III
Committee Members,	William H. Neill
	Christopher A. Bailey
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ABSTRACT

Replacement of Fishmeal with Plant Feedstuffs in the Diet of Red Drum

Sciaenops ocellatus: An Assessment of Nutritional Value.

(May 2012)

Joseph Dale Moxley, B.A. Marketing, Angelo State University

Chair of Advisory Committee: Dr. Delbert M. Gatlin III

The expansion of aquaculture has increased demand for fishmeal supplies around the world; this, in turn, has resulted in dramatic increases in the cost of fishmeal, which has sparked interest in alternative feedstuffs. The development of new processing technologies, as well as the expanding generation of by-products from ethanol production has resulted in the development of novel protein sources that have the potential for replacing fishmeal in aquafeeds. The present study assessed the nutritional value of soy protein concentrate (SoyPC), barley protein concentrate (BarPC) and corn protein concentrate (CornPC) in the diet of red drum. Three sequential feeding trials were conducted; in these 50%, 75%, or 90% of the protein provided by Special Select™ menhaden fishmeal in the reference diet was replaced with either SoyPC, BarPC, or CornPC in isonitrogenous (40% CP), isoenergetic (3.1kcal g^{-1}) diets. Red drum with an average weight of 2.5 g, 1.6 g, and 1.5 g for trials 1, 2, and 3, respectively, were stocked in a recirculating system and fed twice daily at a rate approaching apparent satiation for 6 to 8 weeks. Along with the substitution of the selected plant feedstuffs, supplementation of DL-methionine and L-lysine was provided to exceed the established requirements of red drum for lysine and methionine, and glycine was added for palatability. Performance parameters of weight gain, feed efficiency, survival, hepatosomatic index, intraperitoneal fat ratio, and apparent digestibility coefficients for protein along with proximate composition of whole-body tissues were determined in the various trials. Results showed that 50% replacement of fishmeal protein by each of the protein concentrates produced fish performance, condition indices, and whole-body composition similar to those produced by the reference diet. However, replacing 75% and 90% of fishmeal protein with each of the plant protein concentrates reduced fish performance but not as severely as replacing all of the fishmeal protein with equal (33%) contributions from SoyPC, BarPC, and CornPC. Contrarily, these dietary substitutions did not reduce the apparent protein digestibility of the experimental diets. Based on the various results of this study, SoyPC, BarPC, and CornPC can readily replace 50% of the protein provided by menhaden fishmeal without adversely affecting the performance of cultured red drum.

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INTRODUCTION

Aquaculture is one of the fastest-growing food-producing industries in the world. According to the United Nations Food and Agriculture Organization, this sector has experienced an average annual growth rate of 6.6% per year over the past several decades. Excluding aquatic plants, total production in 1950 was less that 1 million tons per year; by 2008, total production had increased to 52.5 million tons with a value of $98.4 billion (FAO 2010).

Aquaculture traces its beginnings back 4000 years, to China during 2000-1000 B.C., with the culture of common carp *Cyprinus carpio.* Throughout subsequent times, aquatic-animal husbandry methods and practices were developed to expand aquaculture around the world. Today, a variety of culture systems operating at various levels of production intensity are used for commercial production of a diverse group of herbivorous, omnivorous, and carnivorous fishes (Rabanal 1988).

Intensive aquaculture

Intensive aquaculture relies heavily on managed environmental conditions to maximize production. Filtration, aeration, and the input of exogenous feeds, including manufactured feeds, are all components of intensive culture systems. The success of high-production intensive culture relies heavily on nutritionally replete diets, tailored to the individual species being cultured.

This thesis follows the style of *Aquaculture Nutrition*.

Fishmeal as an ideal protein feedstuff for fish

It is known that fish generally require a higher level of protein in their diet compared to terrestrial animals (Lovell 1991). Fishmeal has traditionally been the most nutritious feedstuff provided in prepared aquafeeds. It has a high level of crude protein usually on the order of 60 to 72% of dry weight. Fishmeal, when included in fish feeds provides superior palatability, digestion, and absorption. Along with its well-balanced amino acid profile and high lipid content, fishmeal generally provides adequate nutrition for optimal fish growth and health. Due to its high nutrient digestibility, the use of fishmeal in prepared feeds generally reduces the amount of wastes produced by the fish, making it an important constituent in intensive aquaculture systems (Miles & Chapman 2006).

Fishmeals are typically derived from capture and reduction fisheries and are one of the most nutritious feedstuffs for fish. In 2006, the aquaculture sector consumed approximately 3.7 million tons of fishmeal and 0.84 million tons of fish oil (Tacon & Metian 2008). However, with the growth of aquaculture, the supplies of fishmeal and fish oil are becoming more limited thus increasing their costs. New and Wijkstroem (2002) estimated that by the year 2015, the commercial aquaculture industry is expected to utilize nearly 4.6 million tons of fishmeal and 1.9 million tons of fish oil. This continued expansion of intensive aquaculture requires substitutes for fishmeal to be developed by utilizing different protein resources such as animal by-products, plant feedstuffs, and other novel protein feedstuffs (Naylor *et al*. 2009).

Alternatives to fishmeal

Animal by-products. One alternative to fishmeal is a variety of animal by-products. Terrestrial animal by-products have been used in fish feeds for many decades. Examples include liver and spleen as well as meat and bone meal, produced as remnants from the processing of swine, cattle and horses. Poultry by-products include rendered parts of poultry carcasses such as bones, offal and undeveloped eggs. Feather meal is another product rendered from poultry which is hydrolyzed with heat, pressure and then ground and dried into a meal. Blood meal, processed into a dried powder, is usually rendered from cattle as another animal processing by-product (Bureau 2006). These various animal by-products have been used to replace some portion of fishmeal in feeds for various aquatic species; however, their use is prohibited in the European Union in contrast to the Americas and Asia due to concerns about potential disease transmission (Naylor *et al*. 2009). Thus, these animal by-products are not anticipated to relieve a considerable amount of the fishmeal demand on a global scale.

Plant feedstuffs. Another group of alternatives to fishmeal is a wide range of different plant-derived feedstuffs. Examples of plant feedstuffs available for use in aquafeeds include but are not limited to, products derived from the processing of wheat, barley, soybean, corn, cotton seed, peas/lupins, as well as distillers dried grains generated after ethanol production from various cereal grains.

When selecting plant protein feedstuffs for aquafeeds there are several key characteristics to consider. Candidates must possess adequate nutritional value, be available in ample supply at reasonable costs, and have adequate physical characteristics for feed manufacturing purposes. Plant feedstuffs usually contain more carbohydrates and fiber than animal-derived feedstuffs which may lead to higher fecal excretion and waste production by the organisms fed diets containing such feedstuffs. Refined protein feedstuffs from plants, which contain lower levels of fiber and starches, will have reduced levels of insoluble carbohydrates (Naylor *et al*. 2009). Various chemical and physical means such as ethanol extraction and air classification have been used to remove undesirable components such as oligosaccharides and fiber, respectively, from certain plant feedstuffs.

Anti-nutritional factors associated with plant feedstuffs

Plant feedstuffs may contain certain anti-nutrients which can have a negative impact on cultured organisms. Many of these compounds are produced by the plant to protect itself from natural predators. Anti-nutrients or anti-nutritional factors may be defined as “those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms, for example, inactivation of some nutrients, and diminution of the digestive process or metabolic utilization of feed, which exerts effects contrary to optimum nutrition” (Akande *et al*. 2010). Anti-nutrients that may be present in certain plant feedstuffs and established to adversely affect aquatic organisms include phytic acid, trypsin and other protease inhibitors, gossypol, lectins, saponins, tannins, alkaloids, antigenic compounds, cyanogens, thiocyanate, mimosine, cyclopropenoid fatty acids, canavanine, antivitamin factors, and phorbol esters (Francis *et al*. 2001; NRC 2011).

Nutrient limitations of plant feedstuffs

Along with anti-nutritional factors, plant feedstuffs may be deficient in certain nutrients that are needed by fish. Previous research has established that all fish species require 10 indispensable amino acids (IAAs) in their diet (Craig & Helfrich 2002). Plant feedstuffs may lack adequate concentrations of certain IAAs as well as essential fatty acids required for optimal growth. Optimization of the amino acid profile of aquafeeds, while seeking to replace fishmeal, is essential to increase the efficiency of fish production. Thus, diet formulations containing large quantities of some plant feedstuffs may require the supplementation of certain limiting amino acids (Gatlin *et al*. 2007). The present study is focused on some of the most refined, high-protein plant feedstuffs currently available. Such feedstuffs will be described below along with their nutritional profiles.

Plant feedstuffs used in this study

Soybean meal and soy protein concentrate. Soybean meals are important ingredients for fish feeds based upon their relatively high levels of crude protein [35 to 40% for full-fat meals, 45 to 50% for defatted (solvent-extracted) meals, and 65 to 80% for soy protein concentrates], as well as their reasonably well balanced amino acid profiles (Storebakken *et al*., 2000). When compared with fishmeal, the amino acid composition of soybean meals is severely limiting in the sulfur amino acids methionine and cysteine.

Thus, methionine or total sulfur amino acid requirements of fish, especially carnivorous fish may not be met by replacing large amounts of soybean products for fishmeal in diets. Soybean meals also have lower fat and ash levels then fishmeal, thus requiring dietary supplementation with proper mineral premixes and lipids to help overcome this constraint (Gatlin *et al*. 2007).

Barley protein concentrate. Recent advances in the dehulling of barley as well as fermentation processes have allowed the development of protein concentrates which appear to be attractive candidates for aquaculture feeds. Fermentation processes can produce barley protein concentrates with approximately 60% crude protein by weight, which are both highly digestible and palatable to fish (IBC 2010). While having a balanced nutritional profile, barley also has a reduced level of anti-nutritional factors as compared to some other plant ingredients (IBC 2010).

Corn protein concentrate. Corn protein concentrate is produced using a wet milling technique to extract and separate the endosperm of the corn kernel. Once refined, this feedstuff may contain up to 80% crude protein and less that 1% starch on a dry-weight basis (AAFCO 2007). However, corn protein concentrate is deficient in lysine, as are all corn products. Thus, supplementation with lysine will typically be required for most fish feeds that contain relatively high levels of corn products. However, corn protein concentrate is rich in the sulfur-containing amino acids (Phillips & Sternberg 1979) and thus may be blended with soy protein concentrate to achieve a more balanced amino acid profile.

Red drum aquaculture

The red drum *Sciaenops ocellatus* is a carnivorous species that will be the focus of this evaluation as a model for marine fish aquaculture. Culture of red drum began in the late 1970s, motivated by seafood-market shortages of this species owing to prohibition of commercial fishing on dwindling wild stocks in the Gulf of Mexico. Methods have been developed, particularly in Texas and Florida, for controlled reproduction and hatchery rearing of juvenile fish to aid in enhancing wild stocks. Reliable techniques in aquaculture also have increased commercial production of red drum as a food fish worldwide, which has risen from 2,232 tons in 2001 to 53,511 tons in 2008 (FAO 2011). Red drum has proven to be a viable candidate for large-scale aquaculture based upon their ease of captive breeding, production of large quantities of eggs and larvae, and rapid growth (Gatlin, 2000), as well as tolerances to a wide range of salinities and temperatures (Tomasso, 2000a; 2000b).

Red drum has been used to evaluate several different protein feedstuff substitutions in the past. McGoogan and Reigh (1996) and Gaylord and Gatlin (1996) evaluated the digestibility of various plant and animal by-products with red drum. Low ash meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal have all been evaluated in the diet of red drum (Kureshy *et al*. 2000). Along with animal by-products, plant feedstuffs have been incorporated in the diets of red drum. Previous studies also have demonstrated the possibility of incorporating relatively high levels of soybean meal in the diet of red drum. McGoogan and Gatlin (1997) observed the palatability of diets containing soybean meal in place of fishmeal was reduced and practical diets required a minimum of 10% of the protein provided by fishmeal. Further evaluation of soybean feedstuffs with red drum confirmed that a supplementation of sulfur amino acids is required for optimal growth (Davis *et al*. 1995). However, some of the newly developed plant protein feedstuffs have not been thoroughly evaluated with red drum.

Objectives

The present study was conducted to evaluate different plant feedstuffs in the diet of red drum. This was accomplished by analyzing fish performance parameters such as weight gain, survivability, feed efficiency, digestibility, and whole-body composition of fish fed various diets in which different plant feedstuffs were incrementally substituted for menhaden fishmeal.

METHODS

Experimental diets

Three feeding trials were conducted to evaluate different plant feedstuffs. Each feeding trial was of 6 to 8 weeks duration. The diets in each trial were formulated to contain 40% crude protein, as well as 10% lipid and 3.1 kcal digestible energy per gram. Some diets were supplemented with methionine and/or lysine to avoid potential amino acid deficiencies, and glycine was included in all diets in trials 2 and 3 to increase palatability (McGoogan & Gatlin 1997). All diets were supplemented with a mineral premix as well as a vitamin premix to meet or exceed the established nutritional requirements of red drum and other warmwater species (NRC 2011).

The reference diet in each trial was formulated to contain all of its protein from Special Select™ menhaden fishmeal (Omega Protein, Houston, TX). Experimental diets were formulated in which the various plant feedstuffs replaced incremental amounts of protein provided by menhaden fishmeal in the reference diet. Protein replacement levels ranged from 50 to 90% of that provided by menhaden fishmeal. Four different plant feedstuffs were evaluated including, dehulled, solvent-extracted soybean meal (SBM), soy protein concentrate (SoyPC), barley protein concentrate (BarPC) and corn protein concentrate (CornPC).

All diets were prepared by mixing dry ingredients in a V-mixer. The dry mixture was then homogenized with oil and water using an industrial mixer with a meat grinding attachment, and pelleted with a 3-mm die. Resulting pellets were dried for 24 h using forced air at 25°C. The diets were analyzed in duplicate using AOAC (1990) procedures for crude protein, lipid and ash contents.

Trial 1. This trial evaluated ten diets (reference and nine test diets) over a 6-week period. The ingredient composition for each diet is summarized in Tables 1 and 2. Each diet was formulated to substitute 90% of the fishmeal protein in the reference diet with plant feedstuffs; one additional dietary treatment was formulated with BarPC at 50% replacement of fishmeal protein. In addition, the potential benefit of amino acid (AA) supplementation of the plant-based diets with both lysine and methionine was evaluated by comparing the responses of fish fed each diet with or without AA supplementation.

Trial 2. This trial evaluated five diets (reference and four test diets) over a 6-week period. The ingredients used for each diet in trial 2 are summarized in Table 3. Each diet was formulated to substitute 50% or 75% of the fishmeal protein in the reference diet with SoyPC and BarPC. AA supplementation of both lysine and methionine was provided in each experimental diet as well glycine for added palatability.

Trial 3 The last trial evaluated five diets (reference and four test diets) over an 8-week period. The ingredients used for each diet in trial 3 are summarized in Table 4. Three diets were formulated to substitute 50% of the fishmeal protein in the reference diet with that from either BarPC, SoyPC, or CornPC. One additional dietary treatment was

Table 1 Diet composition (g kg^{-1} dry weight) of the experimental diets for Trial 1.

Ingredient	Diet Designation[1]				
	Reference	**SoyPC 90%**	**SoyPC 90%+AA**	**BarPC 50%+AA**	**BarPC 90%**
Menhaden fishmeal	594.2	59.4	59.4	297.2	59.4
CornPC	-	-	-	-	-
BarPC	-	-	-	375.4	675.8
SoyPC	-	511.2	511.2	-	-
SBM	-	-	-	-	-
Dextrinized starch	157.5	157.5	157.5	157.5	157.5
Menhaden oil	52.5	92.0	92.0	26.2	15.8
Vitamin premix	30.0	30.0	30.0	30.0	30.0
Mineral premix	40.0	40.0	40.0	40.0	40.0
Carboxymethyl cellulose	21.6	21.6	21.6	21.6	21.6
Lysine HCl	-	-	5.0	5.0	-
DL-Methionine	-	-	1.2	1.2	-
Celufil	149.2	231.1	109.1	100.7	111.0
Analyzed proximate composition (g/kg dry weight)[2]					
Dry matter	873.0	882.0	894.0	890.0	803.0
Protein	429.0	438.0	429.0	434.0	418.0
Lipid	71.0	71.0	70.0	70.0	70.0
Ash	70.0	45.0	60.0	110.0	160.0

[1]Refers to the specific plant feedstuffs (SoyPC=soy protein concentrate, BarPC=barley protein concentrate, SBM = soy bean meal, CornPC = corn protein concentrate).
[2]Means of two replicate determinations.

formulated with all three of the plant feedstuffs (AP) to replace 100% of the fishmeal protein. The AP diet was formulated with 33% of each of the three plant feedstuffs previously mentioned. Supplementation of both lysine and methionine was provided in each experimental diet as well as glycine for added palatability.

Table 2 Diet composition (g kg^{-1} dry weight) of the experimental diets for Trial 1.

Ingredient	Diet Designation[1]				
	BarPC 90%+AA	**SBM 90%**	**SBM 90%+AA**	**CornPC 90%**	**Corn 90%+AA**
Menhaden fishmeal	59.4	59.4	59.4	59.4	59.4
CornPC	-	-	-	467.6	467.6
BarPC	675.8	-	-	-	-
SoyPC	-	-	-	-	-
SBM	-	735.3	735.3	-	-
Dextrinized starch	157.5	82.8	82.8	157.5	157.5
Menhaden oil	15.8	75.1	75.1	68.5	68.5
Vitamin premix	30.0	30.0	30.0	30.0	30.0
Mineral premix	40.0	40.0	40.0	40.0	40.0
Carboxymethyl cellulose	21.6	21.6	21.6	21.6	21.6
Lysine HCl	5.0	-	5.0	-	11.5
DL-Methionine	2.5	-	1.2	-	-
Celufil	45.1	6.4	-	20.9	197.4
Analyzed proximate composition (g/kg dry weight)[2]					
Dry matter	892.0	889.0	873.0	921.0	925.0
Protein	428.0	432.0	432.0	422.0	404.0
Lipid	71.0	78.0	74.0	84.0	75.0
Ash	94.0	43.0	82.0	93.0	58.0

[1]Refers to the specific plant feedstuffs (SoyPC=soy protein concentrate, BarPC=barley protein concentrate, SBM=soybean meal, CornPC = corn protein concentrate).

[2]Means of two replicate determinations.

Table 3 Diet composition (g kg^{-1} dry weight) of the experimental diets for Trial 2.

Ingredient	Diet designation[1]				
	Reference	**SoyPC 50%**	**SoyPC 75%**	**BarPC 50%**	**BarPC 75%**
Menhaden fishmeal	621.2	310.6	155.3	310.6	155.3
BarPC	-	-	-	375.4	563.1
SoyPC	-	284.0	425.9	-	-
Dextrinized starch	146.8	144.4	143.2	135.1	129.3
Menhaden oil	33.0	65.4	81.7	23.1	18.2
Vitamin premix	30.0	30.0	30.0	30.0	30.0
Mineral premix	40.0	40.0	40.0	40.0	40.0
Carboxymethyl cellulose	21.6	21.6	21.6	21.6	21.6
Glycine	10.0	10.0	10.0	10.0	10.0
Lysine HCl	-	2.0	3.0	9.6	14.5
DL-Methionine	-	0.7	1.1	3.5	5.2
Celufil	160.6	135.1	122.4	100.2	69.8
Analyzed proximate composition (g/kg dry weight)[2]					
Dry matter	929.0	925.0	928.0	910.0	927.0
Protein	432.0	432.0	437.0	429.0	432.0
Lipid	89.0	87.0	91.0	73.0	85.0
Ash	149.0	93.0	93.0	88.0	80.0

[1]Refers to the specific plant feedstuffs (SoyPC=soy protein concentrate, BarPC=barley protein

[2]Means of two replicate determinations.

Table 4 Diet composition (g kg^{-1} dry weight) of the experimental diets for Trial 3.

Ingredient	Diet designation[1]				
	Reference	**CornPC 50%**	**BarPC 50%**	**SoyPC 50%**	**AP 100%**
Menhaden fishmeal	630.0	315.4	315.4	315.4	-
CornPC	-	260.9	-	-	173.8
BarPC	-	-	328.2	-	254.9
SoyPC	-	-	-	289.8	193.2
Dextrinized starch	153.1	153.1	153.1	153.1	142.2
Menhaden oil	33.0	39.0	22.0	60.0	48.0
Vitamin premix	30.0	30.0	30.0	30.0	30.0
Mineral premix	40.0	40.0	40.0	40.0	40.0
Carboxymethyl cellulose	21.6	21.6	21.6	21.6	21.6
Glycine	10.0	10.0	10.0	10.0	10.0
Lysine HCl	-	10.6	7.4	2.1	13.4
DL-Methionine	-	1.1	3.1	3.5	4.9
Celufil	143.2	173.6	83.5	121.9	120.9
Analyzed proximate composition (g/kg dry weight)[2]					
Dry matter	908.0	906.0	900.0	901.0	899.0
Protein	451.0	458.0	442.0	438.0	430.0
Lipid	119.0	129.0	126.0	112.0	136.0
Ash	137.0	115.0	90.0	108.0	43.0

[1]Refers to the specific plant feedstuffs (SoyPC=soy protein concentrate, BarPC=barley protein concentrate, CornPC=corn protein concentrate, AP=100% replacement with all thee plant feedstuffs).
[2]Means of two replicate determinations.

Fish and feeding trials

The feeding trials took place at the Texas A&M Aquacultural Research and Teaching Facility. Red drum were obtained from the Sea Center Texas Marine Aquarium, Fish Hatchery and Nature Center operated by Texas Parks and Wildlife Department in Lake Jackson, TX. All fish were fed a conditioning diet consisting of the fishmeal reference diet for 1 week prior to the start of each trial. Each trial was conducted in 38-L aquaria connected as a recirculating system, whereby waste water gravity flowed to a settling chamber, then to a biofilter and was pumped through a sand filter before being returned to the aquaria. Water quality was maintained within acceptable levels for red drum. Synthetic sea water was prepared using well water mixed with stock salt and Fritz brand synthetic sea salts to provide culture water of 5-7 ppt salinity. Water temperature was maintained at $26\pm2^{\circ}C$ by conditioning ambient air. Dissolved oxygen was maintained at close to air saturation using compressed air and diffused through air stones. The fish were subjected to a 12h light:12h dark photoperiod using fluorescent lights controlled by timers.

In trial 1, a total of 25 fish (mean weight 2.5 ± 0.5 g/fish) were stocked into each aquarium and the ten diets were assigned to three replicate aquaria per treatment. In trial 2, a total of 25 fish (mean weight 1.6 ± 1.0 g/fish) were stocked into each aquarium and the five diets were assigned to three replicate aquaria per treatment. Finally, in trial 3, a total of 20 fish (mean weight 1.5 ± 0.5 g/fish) were stocked into each aquarium and the five diets were assigned to three replicate aquaria per treatment. At the start of each trial, 10 fish were collected from the remaining population and frozen for subsequent analysis of whole-body composition.

After a 1-week conditioning period in each feeding trial, the reference and experimental diets were fed to triplicate groups of juvenile red drum at a rate approaching apparent satiation. Fish were fed twice daily, 7 days per week. At the end of each week, fish in each aquarium were collectively weighed and feeding rate adjusted accordingly. Over the course of each feeding trial, feeding rate ranged from 3 to 7% of body weight per day depending on fish size. Each week the feeding rate was maintained at the same level for all dietary treatments and adjusted to approach apparent satiation without overfeeding.

Sample collection and analysis

At the end of each trial, three fish per tank were collected from each aquarium and euthanized with an overdose of MS-222 at 150 mg/l. Composite samples of three fish per aquarium were homogenized and subjected to proximate analysis to determine crude protein, lipid, moisture, and ash in whole-body tissue (AOAC 1990). Three additional fish per aquarium were euthanized, weighed and then dissected to obtain liver and intraperitoneal fat (IPF) weights for computing hepatosomatic index (HSI) and IPF ratio values. Performance parameters measured were weight gain, survival, and feed efficiency. Along with these performance parameters then apparent digestibility coefficient of protein in the experimental diets was analyzed for trial 3 (Gaylord &

Gatlin 1996). Data was subjected to analysis of variance and means separated using Tukey's test. Treatment effects were considered significant at $P \leq 0.05$.

RESULTS

Trial 1

Performance parameters. Red drum fed the experimental diets in trial 1 exhibited widely varying performance (Table 5). Fish fed the reference diet had the greatest weight gain which was statistically similar only to fish fed BarPC at 50% replacement. Amongst the 90% replacement diets, the highest weight gain was obtained by fish fed the SBM diet, with those fed the SBM diet supplemented with AAs being statistically similar to that of fish fed the BarPC 50% and 90% replacement diets supplemented with AAs. All diets with the supplementation of AAs supported significantly increased weight gain of red drum compared to their respective non-supplemented diets. Increases expressed as a percent of the non-supplemented diets were 294, 98, 76, and 45% for CornPC, SBM, BarPC, and SoyPC, respectively.

Feed efficiency of fish fed the reference diet was 0.72, which was statistically similar to that of fish fed the 50% BarPC diet at 0.75 and the SBM supplemented with AAs at 0.62. Increases in feed efficiency were observed for fish fed the diets with AA supplementation compared to those without supplementation. Increases in feed efficiency expressed as a percent of the non-supplemented groups were 41, 74, 98, and 117% for SoyPC, SBM, CornPC, and BarPC, respectively.

Table 5 Weight gain, feed efficiency, survival, and condition indices of red drum fed diets containing different protein feedstuffs in Trial 1[1].

Experimental diet	Weight gain (% of initial wt.)	Feed efficiency (g gain/g dry feed)	Survival (%)	HSI[4,5]	IPF ratio[4,6]
1. Menhaden fishmeal (reference)	677[a]	0.73[a]	53[ab]	2.7[ab]	1.0
2. Soy protein concentrate 90% subst.	170[de]	0.29[cde]	30[b]	1.1[c]	0.2
3. Soy protein concentrate 90% subst.[2] + AA[3]	246[de]	0.40[bcd]	56[ab]	1.8[abc]	0.4
4. Barley protein concentrate 50% subst. + AA	577[ab]	0.75[a]	89[a]	3.2[a]	0.8
5. Barley protein concentrate 90% subst.	166[de]	0.22[de]	39[b]	1.8[abc]	0.4
6. Barley protein concentrate 90% subst. + AA	291[cd]	0.47[bc]	47[ab]	2.2[abc]	0.2
7. Soybean meal 90% subst.	235[de]	0.36[cde]	42[b]	1.5[bc]	0.3
8. Soybean meal 90% subst. + AA	465[bc]	0.62[ab]	45[ab]	1.9[bc]	0.7
9. Corn protein concentrate 90% subst.	38[e]	0.13[e]	64[ab]	1.2[bc]	0.4
10. Corn protein concentrate 90% subst. + AA	151[de]	0.26[cde]	30[b]	2.2[abc]	0.2
ANOVA					
Pr > F[7]	0.0001	0.0001	0.0067	0.0023	0.09
Pooled SE	41.51	0.05	0.09	0.3	0.21

[1]Means of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$.

[2]Replacement percent of fishmeal protein.

[3] AA = amino acid supplementation, specifically to provide lysine 2.5% of diet and total sulfur amino acids 1.3% of diet.

[4] Means of three individual fish from each of three replicate tanks.

[5] Hepatosomatic index = liver weight * 100/total fish weight.

[6] Intraperitoneal fat ratio = total IPF weight * 100/total fish weight.

[7] Significance probability associated with the F statistic.

Survival of fish in trial 1 was not particularly high compared to values normally obtained with red drum in this laboratory. However, no disease-causing organisms were identified during the trial. Fish fed the reference diet had 53% survival which was statistically similar to that of fish fed the 50% BarPC diet at 89%. Fish fed the plant feedstuffs at 90% substitution had the lowest survival. There was a general tendency for survival to increase for fish fed the plant-based diets with supplementation of AA except for fish fed the CornPC diet.

Condition indices. The HSI varied significantly among fish fed the various diets while the IPF ratio did not (Table 5). Fish fed the reference diet had the second highest HSI value at 2.7 which was statistically similar to fish fed the 50% BarPC diet, which had the highest value of 3.2. The lowest HSI values were associated with the four lowest-preforming diets of 90% SoyPC, 90% SBM, and 90% CornPC without amino acid supplementation as well as the 90% SBM diet supplemented with AAs. Fish fed all diets with the supplementation of AAs had increased HSI values, which was primarily due to the increased growth responses associated with AA supplementation.

Whole-body composition. Proximate composition of whole-body tissues from fish fed the various diets was not affected by the dietary treatments to any appreciable extent except for moisture (Table 6). Moisture content of fish fed the diets varied significantly with those fed the reference diet having the lowest percent moisture at 73.8% and those fed the CornPC 90% replacement diet having the highest moisture level at 77.8%. Moisture values of fish fed the other diets were intermediate and statistically similar. Fish fed the reference diet had the highest percent protein level amongst all treatments at 22.4%; however, there were no significant differences among any of the other treatments. Fish fed the reference diet had the second highest percent lipid level at 4.3%, but once again there were no significant differences among any of the other treatments. Ash content of fish fed the various diets ranged from 3.6 to 4.7% with no significant differences among any of the treatments.

Table 6 Proximate composition (% of fresh weight) of whole-body tissues of red drum fed diets containing different protein feedstuffs in Trial 1[1].

Experimental diet	Protein	Lipid	Ash	Moisture
1. Menhaden fishmeal (reference)	22.4	4.3	4.1	73.8[b]
2. Soy protein concentrate 90% subst.[2]	16.5	1.9	4.5	76.7[ab]
3. Soy protein concentrate 90% subst. + AA[3]	17.8	3.0	4.0	76.3[ab]
4. Barley protein concentrate 50% subst. + AA	18.7	4.7	4.2	73.8[b]
5. Barley protein concentrate 90% subst.	18.6	6.4	4.5	76[ab]
6. Barley protein concentrate 90% subst. + AA	18.0	3.8	4.0	75.9[ab]
7. Soybean meal 90% subst.	16.6	2.5	4.3	75.6[ab]
8. Soybean meal 90% subst. + AA	17.0	4.0	3.6	77.5[ab]
9. Corn protein concentrate 90% subst.	16.2	3.2	5.0	77.8[a]
10. Corn protein concentrate 90% subst. + AA	15.8	3.2	3.8	76.7[ab]
ANOVA				
Pr > F[4]	0.25	0.26	0.29	0.018
Pooled SE	1.63	1.06	0.36	0.76

[1]Means of composite samples of three fish from each of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$.

[2] Replacement percent of fishmeal protein.

[3] AA = amino acid supplementation, specifically to provide lysine 2.5% of diet and total sulfur amino acids 1.3% of diet.

[4] Significance probability associated with the F statistic.

Trial 2

Performance parameters. Performance parameters of red drum fed the various diets in trial 2 are summarized in Table 7. Fish fed the reference diet had the second greatest weight gain at 759% but was statistically similar to that of fish fed the BarPC 50% and SoyPC 50% replacement diets. Statistically lower weight gain was observed for fish fed the BarPC 75% and SoyPC 75% replacement diets. Fish fed the reference diet had the second highest feed efficiency at 0.78 but it was statistically similar to that of the fish fed all other diets except the SoyPC 75% replacement diet. Survival of fish fed that various diets ranged from 52 to 69% with no statistical significance observed among treatments. Following completion of the feeding trial these fish were donated to another research facility; thus, there were no condition indices and whole body composition data for trial 2.

Table 7 Weight gain, feed efficiency, and survival of red drum fed diets containing different protein feedstuffs in Trial 2[1].

Experimental diet	Weight gain (% of initial wt.)	Feed efficiency (g gain/g dry feed)	Survival (%)
1. Menhaden fishmeal (reference)	759[a]	0.78[a]	52.0
2. Barley protein concentrate 50% subst.[2] + AA[3] + Gly[4]	783[a]	0.81[a]	56.0
3. Barley protein concentrate 75% subst. + AA + Gly	497[bc]	0.68[ab]	69.3
4. Soy protein concentrate 50% subst. + AA + Gly	684[ab]	0.77[ab]	57.3
5. Soy protein concentrate 75% subst. + AA + Gly	418[c]	0.55[c]	53.3
ANOVA			
Pr > F[5]	0.0008	0.0001	0.6651
Pooled SE	46.92	2.19	8.82

[1]Means of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$.

[2] Replacement percent of fishmeal protein.

[3] AA = amino acid supplementation, specifically to provide lysine 2.5% of diet and total sulfur amino acids 1.3% of diet.

[4] Glycine added for palatability.

[5] Significance probability associated with the F statistic.

Trial 3

Performance parameters. Red drum fed the reference diet had similar weight gain as fish fed SoyPC 50%, CornPC 50%, and BarPC 50% replacement diets (Table 8). However, statistical separation did occur between fish fed the SoyPC 50% replacement diet which was higher than that of fish fed both the CornPC 50% and BarPC 50% replacement diets. The all plant diet, AP 100%, supported the lowest weight gain which was statistically different than all other treatments.

Table 8 Weight gain, feed efficiency, survival, condition indices, and apparent digestibility coefficient (ADC) of dietary protein for red drum fed diets containing different protein feedstuffs in Trial 3[1].

Experimental diet	**Weight gain** (% of initial wt.)	**Feed efficiency** (g gain/g dry feed)	**Survival** (%)	**HSI**[5,6]	**IPF ratio**[5,7]	**ADC Protein** (%)
1. Menhaden fishmeal (reference)	1894^{b}	0.87^{bc}	86.7	1.7^{c}	0.6	70.6^{b}
2. Corn protein concentrate 50% subst.[2]+AA[3]+Gly[4]	1661^{c}	0.95^{ab}	95.0	2^{bc}	0.7	80.3^{a}
3. Barley protein concentrate 50% subst.+AA+Gly	1709^{bc}	0.92^{abc}	93.3	2.5^{ab}	0.7	76.9^{ab}
4. Soy protein concentrate 50% subst.+AA+Gly	2112^{a}	1.03^{a}	91.7	1.7^{c}	0.6	76.7^{ab}
5. All Plant (33% Corn PC, Barley PC, Soy PC)+AA+Gly	990^{d}	0.80^{c}	91.7	2.7^{a}	0.4	76.9^{a}
ANOVA						
Pr > F[8]	0.0001	0.0045	0.49	0.0005	0.81	0.0376
Pooled SE	69.17	0.031	3.25	0.12	0.10	1.76

[1] Means of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$.

[2] Replacement percent of fishmeal protein.

[3] AA = amino acid supplementation, specifically to provide lysine 2.5% of diet and total sulfur amino acids 1.3% of diet.

[4] Glycine added for palatability.

[5] Means of three individual fish from each of three replicate tanks.

[6] Hepatosomatic index (HSI) = liver weight * 100/total fish weight.

[7] Intraperitoneal fat (IPF) ratio = total IPF weight * 100/total fish weight.

[8] Significance probability associated with the F statistic.

Fish fed the reference diet had the fourth best feed efficiency at 0.87, and was statistically similar to that of fish fed the CornPC 50% and BarPC 50% replacement diets as well as the AP 100% replacement diet. The SoyPC 50% replacement diet resulted in the highest feed efficiency of 1.03, which was statistically different from the reference diet but similar to the BarPC 50% and CornPC 50% replacement diets. Survival ranged from 86.7 to 95% and there were no statistical differences among the various treatments.

Apparent digestibility coefficients of the protein in the diets varied considerably. Fish fed the reference diet had the lowest digestibility of 68.3% which was statistically similar to those fed the SoyPC 50% and BarPC 50% replacement diets which were 73.4% and 74.2% respectively. Both the CornPC 50% and AP 100% replacement diets were statistically different than the reference diet at 78.7% and 74.7%, respectively.

Condition indices. Fish fed the reference diet had the lowest HSI value which was statistically similar to that of fish fed the CornPC 50% and SoyPC 50% replacement diets (Table 8). Fish fed the AP 100% diet and BarPC 50% diet had the highest HSI values. There were no statistical differences in IPF ratio among fish fed the various dietary treatments.

Whole-body composition. Proximate composition of the whole-body tissues of fish fed the various diets was only minimally affected with some significant differences in ash and moisture being detected (Table 9). Red drum fed the AP 100% diet had the lowest whole-body ash which was statistically different from all other treatments. Fish fed the reference diet had the lowest whole-body moisture at 75.4% which was statistically different from the other dietary treatments except for the SoyPC 50% diet. Fish fed the AP 100% replacement diet had the highest moisture at 77.7%, which was statistically different from that of fish fed all other experimental diets. No significant differences were detected among any of the dietary treatments with regard to whole-body protein or lipid.

Table 9 Proximate composition (% of fresh weight) of whole-body tissues of red drum fed diets containing different protein feedstuffs in Trial 3[1].

Experimental diet	**Protein**	**Lipid**	**Ash**	**Moisture**
1. Menhaden fishmeal (reference)	68.3	5.7	4.3[a]	75.4[c]
2. Corn protein concentrate 50% subst.[2]+AA[3]+Gly[4]	69.7	5.3	4.1[a]	76.6[b]
3. Barley protein concentrate 50% subst.+AA+Gly	68.7	3.8	4.3[a]	76.5[b]
4. Soy protein concentrate 50% subst.+AA+Gly	68.5	4.7	4.2[a]	75.9[bc]
5. All Plant (33% CornPC, BarPC, SoyPC)+AA+Gly	71.0	4.0	3.1[b]	77.7[a]
ANOVA				
Pr > F [5]	0.1142	0.0851	0.0001	0.0003
Pooled SE	0.84	0.47	0.12	0.21

[1]Means of composite samples of three fish from each of three replicate groups. Values in a row that do not have the same superscript letters are significantly different at $P \leq 0.05$.

[2] Replacement percent of fishmeal protein.

[3] AA = amino acid supplementation, specifically to provide lysine 2.5% of diet and total sulfur amino acids 1.3% of diet.

[4] Glycine added for palatability.

[5] Significance probability associated with the F statistic.

DISCUSSION

This thesis was intended to assess different plant protein feedstuffs as partial replacements for fishmeal in the diet of red drum. Through a series of trials with different inclusion rates of SoyPC, BarPC, and CornPC, various performance parameters and condition indices were evaluated to assess the a relative nutritional value of these high-protein plant feedstuffs for red drum. Starting with substitution levels of 90% of dietary protein from fishmeal in trial 1 proved that performance parameters of weight gain and feed efficiency were severely affected with such a high replacement level. The reduction of plant feedstuffs to replace 75% and 50% of the dietary protein from fishmeal in trial 2 resulted in a trend of increased performance as inclusion rates of each plant feedstuff were reduced. Based upon the results from trial 1 and 2, it became evident that once the replacement of fishmeal protein was limited to 50%, performance parameters of fish fed the experimental diets were comparable to those fed the fishmeal reference diet. Results observed in trial 3 indicated that all of these plant feedstuffs evaluated have potential to be included in the diet of red drum, and possibly replace up to half of the fishmeal protein without significantly reducing growth performance and feed efficiency.

The improvement in performance of red drum fed diets with supplemented AAs in trial 1 is consistent with the understanding that these plant feedstuffs are limiting in certain indispensable AAs, which are crucial to support growth, immunity, and reproduction (Gatlin *et al*. 2007). Previous research with red drum determined that lysine should be provided at 1.77% of the diet (Craig & Gatlin 1992) and total sulfur amino acids (TSAA) should be provided at 1.21% of the diet (Moon & Gatlin 1991) in a 40% crude protein diet to satisfy the minimum requirements for these two most important AAs. In trial 1 of the current study, both lysine as well as TSAA was supplemented at rates that would exceed the known requirements of red drum to determine if supplementation of these AAs was needed for the various plant feedstuffs at the 90% fishmeal protein replacement level. Based upon the results observed in trial 1, it was decided to include the same amounts of lysine and TSAA supplementation to diets in trials 2 and 3 to ensure sufficiency of those two AAs. However, it is important to realize that depending on the inclusion level of these refined plant feedstuffs and other protein feedstuffs, supplementation of such AAs may or may not be required.

In trial 1 SoyPC at a replacement level of 90% provided approximately 1.94% lysine and 0.5% TSAA in the diet. When added to the 10% of protein provided by fishmeal it brought the total amount of lysine to 2.19% and TSAA to 0.62% of diet. Based upon the previously stated AA requirements of red drum, the dietary lysine requirement was surpassed while the dietary TSAA requirement was not met, creating a 0.59% deficit of TSAA in the diet such that TSAA were limiting. In the subsequent trials, the SoyPC inclusion rate was reduced to 75% of the dietary protein in trial 2 and finally 50% in trial 3 which brought about different levels of AAs contributed by this feedstuff in the diet. In trial 3 for example, when the inclusion rate of SoyPC was reduced to provide 50% of dietary protein, the total amount of lysine contributed by the feedstuffs was increased to 2.35% as well as TSAA to 0.88%. Thus, the TSAA were still limiting; but, this margin was reduced and limited the amount of TSAA supplementation needed.

Dietary substitutions of BarPC as well as CornPC resulted in similar trends as SoyPC in that they contributed less lysine and methionine than fishmeal. In trial 1, BarPC at 90% replacement without AA supplementation resulted in a deficiency of 0.38% for lysine and 0.52% for TSAA. Once the inclusion rate was reduced to 50%, lysine was in surplus of the quantified requirement of red drum; however, TSAA were still deficient by 0.29% of diet. For CornPC, in trial 1 with 90% replacement of fishmeal protein, lysine and TSAA were both deficient at 0.85% and 0.16% of diet, respectively. Once the inclusion rates were reduced to 50% of dietary protein, the dietary requirement for lysine was still limiting by .13%, as well as TSAA were marginally deficient by 0.09% of diet. Based upon these results, identification of proper supplementation of AAs is required to offset the potentially adverse effects of replacing fishmeal with plant feedstuffs.

The use of soybean meal in aquafeeds has been well documented in the past. Species respond differently when varied levels of soybean meal are incorporated in the diets of marine fishes (Refstie *et al*. 2000). Juvenile cobia *Rachycentron canadum* were shown to tolerate replacement of fishmeal with solvent-extracted soybean meal up to 40% of diet in a 45% crude protein diet (Chou *et al*. 2004). As with this trial with red drum, reductions of weight gain and feed efficiency occurred as the inclusion level of SBM was increased in the diet. Moreover, Davis *et al*. (1995), reported similar results as this study in which diets replacing large amounts of fishmeal with SBM significantly reduced growth of red drum. This evaluation also concluded that high inclusion rates of SBM in the diet of red drum caused a reduction in palatability. McGoogan and Gatlin (1997) found with red drum that in a 38% crude protein diet replacing 90% of fishmeal protein with SBM did not limit weight gain. However, this is not consistent with the results seen in trial 1 of this study where performance parameters of weight gain and feed efficiency were significantly reduced with such high inclusion rates.

In contrast to SBM which is typically hexane-extracted, SoyPC is further refined using an ethanol extraction technique or enzyme hydrolysis. Once refined, SoyPC has several advantages over SBM including a reduced oligosaccharide content and increased crude protein content. SoyPC also is lower in phytoestrogens and other proteins such as agglutinins and other lectins to reduce potential allergenic effects (Swick 2007). Several studies with freshwater and marine carnivorous fish evaluating SoyPC as a replacement for fishmeal have obtained similar results as the present study, in which up to 50% of the dietary protein provided by fishmeal could be replaced. Mambrini *et al*. (1999) looked at the replacement of fishmeal with SoyPC in the diet of rainbow trout *Oncorhynchus mykiss*. This study determined that a maximum inclusion rate of 50% replacement with SoyPC with the addition of DL-methionine produced optimum growth. Research with other species such as Atlantic halibut *Hippoglossus hippoglossus* concluded that 44% replacement of fishmeal protein by SoyPC resulted in increased feed intake as well as growth rate (Berge *et al*. 1999). Kissil *et al*. (2000) found that feed intake and weight gain were inversely related to inclusion levels of SoyPC in the diets of gilthead seabream

Sparus aurata. Determinations from that study also concluded that the relative palatability of SoyPC could be a limiting factor in their use. Takagi *et al*. (2001) fed diets replacing 52% of the fishmeal protein with SoyPC to red sea bream *Pagrus major*. They found supplementations of both lysine and methionine improved the quality of the diet.

BarPC could replace up to 50% of the protein provided by fishmeal in the diets of red drum based upon results of the present study. However, limited information on BarPC use in aquafeeds has been published to date. Observations of a higher apparent protein digestibility coefficient of 92% for BarPC relative to fishmeal at 90% was reported for rainbow trout suggesting that BarPC is a good candidate for aquafeeds (Gaylord *et al*. 2008). Morken *et al*. (2011) also observed an increase in digestibility when BarPC was incorporated in the diets of rainbow trout, however, digestibility of AA was not affected. Research with other species such as Atlantic salmon *Salmo salar* and Arctic charr *Salvelinus alpinus* also reported relatively high apparent digestibility coefficients for crude protein at 96.3% and 85.1%, respectively (Burr *et al*. 2011). Those values were higher than the values obtained with red drum in the present study for the toatal dietary protein.

CornPC is also a good candidate to replace up to 50% of the protein provide by fishmeal in the diets of red drum. CornPC has been studied only to a limited extent regarding its use in aquafeeds; however, some evaluations have been conducted with corn gluten meal, which is relatively similar to CornPC. These corn protein isolates have been determined to be highly digestible to juvenile cobia, with 94% crude protein digestibility (Zhou *et al*. 2004) and to rainbow trout with 96% crude protein digestibility {Morales *et al*. 1994). Regost *et al*. (1999) also examined corn gluten meal as a replacement for fishmeal and found that it could replace up to 33% of fishmeal in the diet of turbot *Psetta maxima* and that supplementations of lysine improved the performance of the diets. Pereira and Oliva-Teles (2003) also found corn gluten meal to be a valuable alternative and could replace up to 60% of the protein from fishmeal in diets for gilthead sea bream juveniles with no negative effects on fish performance. Finally, Kikuchi (1999) found that corn gluten meal in the diets of Japanese flounder *Paralichthys olivaceus* could replace up to 40% of the protein from fishmeal without adversely effecting growth while inclusion rates of 60% or higher negatively affected weight gain, feed efficiency, and protein efficiency.

Another important aspect relative to evaluating the nutritional value of alternatives to fishmeal is the costs associated with such feedstuffs. One method of doing this is a protein value assessment (Alcock 2004). To determine protein costs, the cost per ton of dry matter for each plant protein feedstuff along with fishmeal were obtained from recently published values. Dividing the per ton cost of each ingredient by its crude protein percentage allowed expression of their cost per unit protein (Table 10).

Table 10 Estimated costs of various protein feedstuffs.

Protein Source	$/ton (dry weight basis)	% Crude protein	Cost per unit protein
Fishmeal, Menhaden[a]	1,300	65	$1.00
Soy protein concentrate[b]	1,100	68	$0.81
Barley protein concentrate[c]	950	60	$0.79
Corn protein concentrate[d]	620	60	$0.52

[a]Price from www.indexmundi.com (10/18/11).

[b]Price from Tianjin Huge Roc Enterprises Co., Ltd. (10/18/11)

[c]Price from (Druham, S. 2010)

[d]Price from Shandong Yuyuan Group Co., Ltd (10/18/11)

As noted in Table 10, the cost of menhaden fishmeal per unit protein is highest followed by SoyPC, BarPC, and finally CornPC. Up until recently fishmeal was the most cost-effect protein source available for aquaculture. However, due to factors such as increased demand globally from aquaculture expansion and weather-related events such as El-Niño that periodically reduce fishmeal supplies; the costs for fishmeal have escalated. In 2006, fishmeal jumped to twice its trading value, and since then has retained this value for nearly half a decade. The cost of fishmeal is not anticipated to decrease and thus has sparked interest in evaluating plant feedstuffs which have retained more stable prices relative to fishmeal (Hardy 2010).

Though plant feedstuffs look to be the more cost-effective protein sources for aquaculture in the future, there are nutritional limits that must be considered. As stated earlier, one major constraint facing most plant feedstuff is their limiting AA profiles. Supplementations of AAs such as methionine and lysine to plant feedstuffs add to the relative cost of the diet. Another economic factor that affects the value of plant feedstuffs as replacements for fishmeal is their anticipated availability. World production of soybeans is expected to increase by 2.2% annually over the next 15 years (Masuda & Goldsmith 2009). CornPC and one of the newest feedstuffs, BarPC, are both anticipated to be available at higher levels in coming years due to the increased demand for ethanol production. Through the ethanol production process, by-products commonly referred to as “distillers dried grains”, are anticipated to rise dramatically in quantity with this increased production (Babcock *et al*. 2008). New enzymatic methods developed by Montana Microbial Products are being used to concentrate the barley protein while using the raw starch for ethanol production (Durham 2010).

SUMMARY AND CONCLUSIONS

As aquaculture continues to grow, so will the demand for high-quality protein feedstuffs to include in prepared feeds. Sustainability of the worldwide aquaculture industry must rely upon alternative protein sources to reduce its reliance on fishmeal, the most nutritious but also the most expensive feedstuff used in fish diets. Development of new technologies and methods of producing high-quality, alternative plant feedstuffs are one way of securing long term sustainability.

Based on results of the present study, the plant feedstuffs SBM, SoyPC, BarPC, and CornPC when supplemented with adequate amounts of AAs are good candidates for partial fishmeal replacement. It was concluded that 50% dietary replacement of fishmeal protein with the three protein isolates allowed red drum to perform similar to that of fish fed a diet with all of its protein coming from fishmeal. Inclusion rates of these plant feedstuffs between 50% and 75% of the protein provided by fishmeal should be evaluated to identify the maximum inclusion level of each individual plant feedstuff. Furthermore, future research should be directed towards blending more than one plant feedstuff to limit the amount of supplemental methionine or lysine required in the diet.

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APPENDIX

Ingredient	Dry Matter	Protein	Lipid	Energy (kcal/g)	Ala	Arg	Asp	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Ser	Thr	Tyr	Val	Pro
Menhaden Fishmeal (Special Select)	933.0	695.0	75.8	57190	47.0	48.0	65.0	97.0	56.0	14.0	28.0	51.0	44.0	21.0	29.0	32.0	32.0	23.0	38.0	39.0
SoyPC	961.0	722.0	0.3	47100	33.0	63.0	90.0	143.0	33.0	19.0	34.0	60.0	39.0	10.0	40.0	45.0	34.0	30.0	40.0	44.0
BarPC	927.0	569.0	58.9	55770	25.0	34.0	35.0	154.0	22.0	12.0	23.0	46.0	18.0	9.0	37.0	30.0	23.0	23.0	35.0	74.0
CornPC	942.0	813.0	41.6	58960	79.0	29.0	54.0	200.0	24.0	18.0	34.0	150.0	15.0	21.0	58.0	54.0	32.0	50.0	45.0	93.0

Appendix A-1 Amino acid composition (g kg^{-1} dry weight) of the various feedstuffs used in trials 1, 2, and 3.

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